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13. SUPPLEMENTARY NOTES

14. ABSTRACT. In the last year we have completed analysis of Phase II of the project. In Phase I we showed that an RNA-based gene signature from a sample taken at sea level could be used to successfully predict in 9 out of 10 individuals who went on to develop acute mountain sickness or who was AMS resistant. In Phase II, results suggest a completely independent sample was equally effective in predicting AMS susceptibility and resistance. These results were presented to the scientific leadership at Fort Detrick last spring. Under the advisement of that review process we have taken an expanded view of the data we are using for predicting AMS. Specifically, in addition to the data collected in this project for Phase I and Phase II, we will add several data sets to the overall analysis. New dataset #1 is analyses of an additional 70+ samples from Phase I. These samples represent subjects who were not very sick or not very well, the middle of the road group. The hypothesis is that a very effective predictive test would predict those who might get very sick or not at all sick, but also those who feel only a little bit impaired by high altitude. New dataset #2 is from a companion project called AltitudeOmics where we have samples from sea level subjects exposed to very high altitude. All of these data will be analyzed in one integrated test to assess effectiveness. Our team of bioinformaticians is working together to realize these analyses in the following year.

15. SUBJECT TERMS
Acute Mountain Sickness Prediction, Hypoxia, High Altitude

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INTRODUCTION:

The goal of this project is to design an easy-to-use cost-effective test that accurately predicts whether or not someone is likely to develop acute mountain sickness (AMS) when they travel to high altitudes.

OVERALL PROJECT SUMMARY:

In this fourth year we were recovering from the slight delays incurred last year due to the long wait for HRPO approval of the second study as well as the replacement of our postdoctoral fellow responsible for the gene expression analyses.

We also expanded our SOW to include the analysis of the remaining 76 samples. We therefore are currently in a NCE period with supplemental budget for the SOW expansion.

QTR 13 Accomplishments (Jan-Mar 2014):
We completed the field study on September 29 2013. 27 research subjects from Dallas successfully completed the high altitude protocols in Breckenridge. We hired a new part-time bioinformatics person to supervise the completely independent analysis of Phase I and Phase II data. The reason for the 'independence' is to avoid the possibility that the research team could influence the results. We started that process in January of 2014 and have completed the initial analyses of Phase I and started the initial de-novo analysis of Phase II.

Dr. Roach also had a chance to present the preliminary data form these studies to the DoD IPR at Fort Detrick. The project was very positively received. The presentation is attached.

QTR 14 Accomplishments (Apr-Jun 2014):
In quarter 14 we hired an additional part-time bioinformatics analyst to assist with the data analysis. That work is going well. Mid-quarter we were asked to prepare a supplementary budget to analyze some additional samples from Phase 1. During this quarter we were still waiting to hear the outcome of that funding request. In the meantime we developed two plans of action for the remaining time on this project, one with no additional funds, and one with the additional funds. Bioinformatics analysis continued throughout this quarter.

QTR 15 Accomplishments (Jul-Sep 2014):
In QTR 15 we got confirmation that we have additional funding which slightly modifies the plan and scope of our work on this project. Our newly hired additional part-time bioinformatics analyst is beginning to assist with the data analysis, and to help develop the plan for the next year of work on AMS Prediction. That work is going well.

QTR 16 Accomplishments (Sep-Dec 2014):
At the end of Q16 we started organizing the frozen RNA samples for the 'extra' analyses. And we began organizing all the additional data we will include in this mega comparison of gene expression for AMS prediction. Work on this project began ramping up during this quarter and will continue to be a major focus for the next 12 months.

Throughout all four quarters of 2014 we have to commit considerable time and resources to continuing IRB review, both locally here in Colorado and at HRPO, which we have done and will continue to do.

KEY RESEARCH ACCOMPLISHMENTS:

- confirmation of our ability based on a sea level test in Phase I to predict acute mountain sickness at high altitude
- design and execution of the Phase II field study
- successful RNA isolation and microarray analyses from the Phase II validation study data
- gaining additional funding to analyze 'middle of the road' samples from Phase I
- addition of data from our companion study, AltitudeOmics, to the database of gene studies to be analyzed for AMS prediction
- expansion of a bioinformatics team to include two additional consultants at no cost to the project
- expansion of the bioinformatics dataset to include advanced clustering analysis which may lead to identification of biological factors linking the AMS prediction signature to biological mechanisms

REPORTABLE OUTCOMES:

Though the initial results are exciting, and verify our original hypothesis, it is premature to proclaim complete success. Today it is accepted practice to validate gene expression findings in an independent cohort before relying on the validity of a gene expression screening test. We are expanding this validation phase to include different samples form higher altitudes, and more samples form Phase I with less severe symptoms. This robust approach to validation will rigorously test whether AMS prediction from a sea level blood test is possible.

CONCLUSION:

If the Phase I expanded sample base, the Phase II validation study and the AltitudeOmics study confirms our initial findings, we are on track to develop a method for predicting risk of AMS in sea level residents. This was the overarching goal of the entire project, and at this point, we are still hopeful to validate the identified gene signature for AMS prediction. This last step has potential to change the way we manage risk in people who have never before gone to high altitude.

Prediction of Acute Mountain Sickness Using a Blood-Based Test

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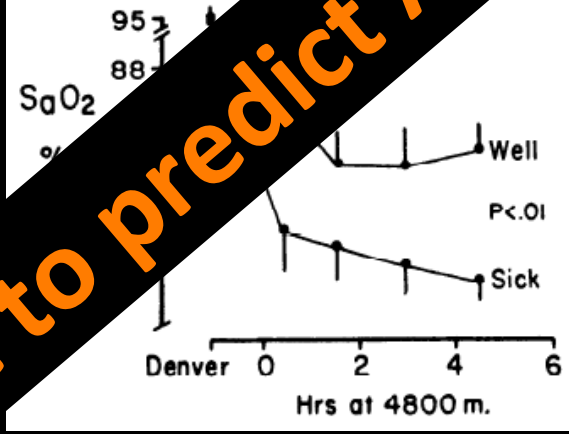
Low acute hypoxic ventilatory response
hypoxic depression in acute altitude

L. G. MOORE, G. L. HARRISON, R. E. Mc CULLOUGH,
A. J. MICCO, A. TUCKER, J. V. WEIL,

Journal of Applied Physiol -12.



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Prediction of Susceptibili n Sickness
by Sa_{O_2} Values during re to Hypoxia

MARTIN BUR MARTIN FAULHABER

ORIGINAL RESEARCH

Arterial Oxygen Saturation for Prediction of Acute Mountain Sickness

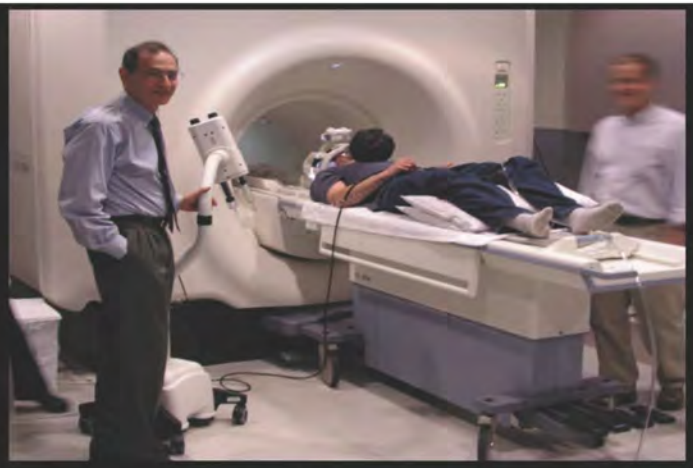
ROACH RC, GREENE ER, SCHOENE RB, HACKETT PH. *Arterial oxygen saturation for prediction of acute mountain sickness.* Aviat Space Environ Med 1998; 69:1182–5.

Physiological Risk Factors for Severe High-Altitude Illness
A Prospective Cohort Study

Jean-Paul Richalet[1,2], Philippe Larmignat[1,3], Eric Poitrine[1], Murielle Letournel[2], and Florence Canouï-Poitrine[4,5]

AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE VOL 185 2012

No physiological test to predict AMS



NIH HL-070362, RR

28 healthy subjects
10 hrs at 4559 m (~16,000 ft)
pre and post hypoxia MRI
perfusion by Gadolinium enhanced imaging

Gene expression measured at low altitude,
before 10hrs at simulated high altitude

paradigm shift

new school:

going ‘fishing’ with new techniques/ technologies married to ‘old school’ sensibilities means you *catch big fish*

hypothesis

unique pattern of gene expression ***predicts*** future AMS

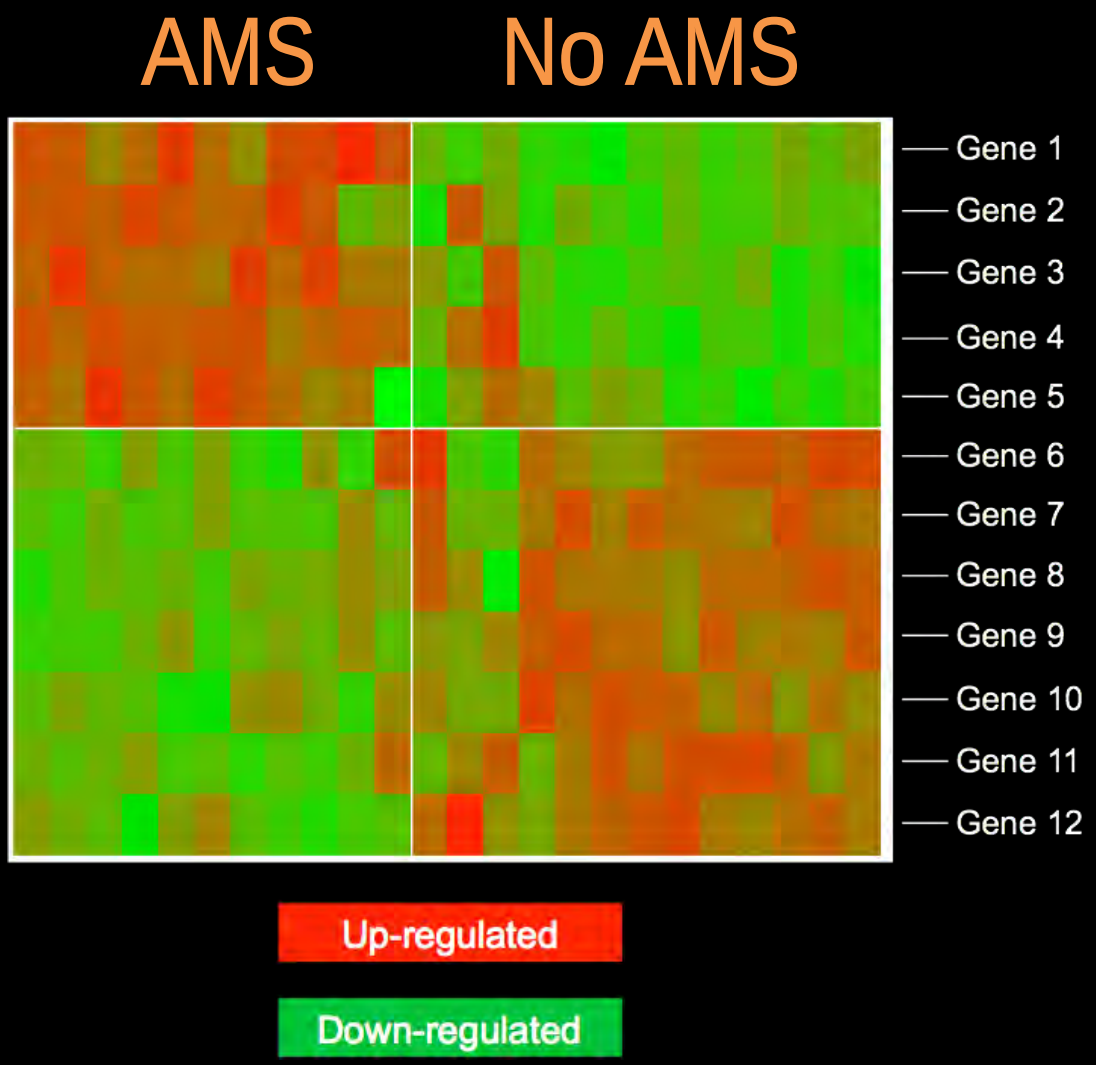


Table 2. Success of 7 Different Statistical Prediction Algorithms

Prediction Classification Method	*Support Vector Machines*	*Compound Covariate Predictor*	*Nearest Centroid*	*Diagonal Linear Discriminant Analysis*	*Bayesian Compound Covariate Predictor*	*3-Nearest Neighbors*	*1-Nearest Neighbor*
Best AMS Prediction Model using expression of 6 mRNAs							
Percent of correct classification of subjects	100%	100%	100%	100%	100%	100%	96%
AMS subjects correctly predicted	14/14	14/14	14/14	14/14	13/14(**1**)	14/14	13/14
Healthy subjects correctly predicted	10/10	10/10	10/10	10/10	10/10	10/10	10/10

(Wilson, Julian and Roach, in review, 2014)

Table 3. Components of the Best AMS Prediction Model

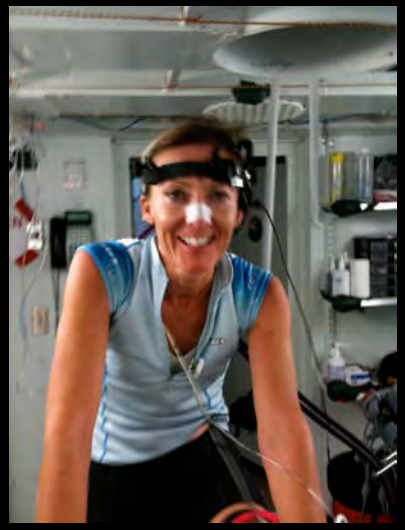
AMS Prediction Classifiers	Description	Affymetrix Probe Set	Parametric p-value	Fold Change From Healthy to AMS
FLJ31306	None	239432_at	2.20E-05	1.61
not named	None	236944_at	3.25E-05	1.58
ERICH1	Glutamate-rich 1	242003_at	4.96E-05	2.24
not named	None	241699_at	5.02E-05	1.55
CALM1	Calmodulin 1	213688_at	9.09E-05	1.73
TLK1	Tousled-like kinase 1	211077_s_at	9.68E-05	2.15

(Wilson, Julian and Roach, in review, 2014)

Potential for AMS Prediction

Blood test kit for pre-ascent prediction of AMS risk

Caveat: must be *validated* in different populations, locations, ascent rates



AMS Prediction

Phase I

Can AMS be predicted by gene expression test, SL to 10K ft?

140 SL subjects, young, healthy, APFT fit

gene exp SL, three days rapid exposure 10Kft (Breckenridge)

Phase II

If Phase I is positive, can it be repeated?

30 healthy subjects, naïve technicians, 2 yrs post Phase I


O'GRIFF
80

9 out of 10 husbands agreed that their wives are always right. The 10th hasn't been seen since the study concluded.

AMS Prediction

Phase II

since Phase I was positive, can it be repeated?

30 healthy subjects, naïve technicians, 2 yrs post Phase I

1 yr delay HRPO

data collection completed late fall 2013

data analysis underway

Future Directions

complete validation analyses ~ 6 months

complete Phase I + Phase II analyses, merging technologies from AltitudeOmics > analysis pipeline ~ 12 months (NCE)

if all tests positive, licensing for widespread distribution

Challenges

cheaper tests, targeted chip

congruence with animal models

generalizability—altitudes reached, starting conditions, specific groups

targets for new understanding pathophysiology

Impact on Warfighters Lives

new AMS prediction test

↑ AMS-prevention

$$ savings

↑ unit performance

↑ treatment cost

↑ evacuation cost

↑ risk: evacuation

↑ risk: unit performance

old status quo